Regulation of gastric and pancreatic lipase secretion by CCK and cholinergic mechanisms in humans

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Gastroenterology Department, Policlinique Médicale Universitaire, Lausanne; Gastroenterology Department, University Hospital, Zurich, Switzerland; Gastroenterology Department, University Hospital, Nijmegen, The Netherlands; and Laboratoire de Lipolyse Enzymatique du Centre National de la Recherche Scientifique, Marseille, France

Borovicka, Jan, Werner Schwizer, Christine Mettraux, Christianna Kreiss, Brigitte Remy, Khaled Asal, Jan B. M. J. Jansen, Isabelle Douchet, Robert Verger, and Michael Fried. Regulation of gastric and pancreatic lipase secretion by CCK and cholinergic mechanisms in humans. *Am. J. Physiol.* 273 (*Gastrointest. Liver Physiol.* 36): G374–G380, 1997.—Gastric lipase (HGL) contributes significantly to fat digestion. However, little is known about its neurohormonal regulation in humans. We studied the role of CCK and cholinergic mechanisms in the postprandial regulation of HGL and pancreatic lipase (HPL) secretion in six healthy subjects. Gastric emptying of a mixed meal and outputs of HGL, pepsin, acid, and HPL were determined with a double-indicator technique. Three experiments were performed in random order: intravenous infusion of *1*) placebo, *2*) low-dose atropine (5 $\mu g \cdot kg^{-} \cdot h^{-1}$), and *3*) the CCK-A receptor antagonist loxiglumide (22 $\mu mol \cdot kg^{-} \cdot h^{-1}$). Atropine decreased postprandial outputs of HGL, pepsin, gastric acid, and HPL ($P < 0.03$) while slowing gastric emptying ($P < 0.05$). Loxiglumide markedly increased the secretion of HGL, pepsin, and acid while distinctly reducing HPL outputs and accelerating gastric emptying ($P < 0.03$). Plasma CCK and gastrin levels increased during loxiglumide infusion ($P < 0.03$). Atropine enhanced gastrin but not CCK release. Postprandial HGL, pepsin, and acid secretion are under positive cholinergic but negative CCK control, whereas HPL is stimulated by cholinergic and CCK mechanisms. We conclude that CCK and cholinergic mechanisms have an important role in the coordination of HGL and HPL secretion to optimize digestion of dietary lipids in humans.

loxiglumide; atropine; pepsin; acid secretion; gastric emptying

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THE EXISTENCE OF GASTRIC lipolysis has been known since the beginning of this century (41). Recently, the cellular origin of human gastric lipase (HGL) has been localized in the chief cells of fundic mucosa (28). HGL has been shown to be responsible for 25% of acyl chain hydrolysis during meal digestion (8). The colocation of HGL and pepsin and their correlated secretory response to pentagastrin stimulation suggested common regulatory mechanisms of these two gastric enzymes (28, 39). Distinct receptors of the two important regulatory hormones, cholecystokinin (CCK) and gastrin, were previously identified in chief cells in the guinea pig stomach (10). Recently, different study groups reported data on the regulation of gastric acid secretion by CCK in dogs and humans (22, 35). By use of specific CCK-A receptor antagonists (loxiglumide, MK-329), these studies showed an inhibitory action of endogenous CCK on gastric acid secretion in contrast to the known stimulatory effect of CCK on pancreatic enzyme secretion in humans (2, 17). Although postprandial CCK-dependent regulatory mechanisms have been studied extensively for the pancreas, no data are available for gastric enzyme secretions in humans.

In this study we therefore applied the cholinergic antagonist atropine and the highly specific CCK-A receptor antagonist loxiglumide (36), which has been shown to inhibit markedly pancreatic enzyme secretion and gallbladder contraction in humans (2). Our aim was to define the physiological role of CCK and cholinergic mechanisms in the regulation of postprandial gastric enzyme secretions in comparison to pancreatic enzyme secretions and to study the relationship between the secretion of HGL and pancreatic lipase (HPL) after a physiological meal in humans.

MATERIALS AND METHODS

Subjects

Studies were performed in six healthy subjects (5 men, 1 woman) between 23 and 30 yr of age and 76 kg mean body weight (range 60–83 kg). All subjects were within 10% of their ideal body weight. No subject was taking any medication, nor did any subject have a history of gastrointestinal symptoms or prior surgery. The studies were approved by the local ethical committee of the University Hospital of Lausanne, and all subjects gave written informed consent.

Experimental Procedure

Double-indicator technique. All studies were performed after an overnight fast. The subjects were intubated with a triple-lumen tube (5 mm OD), with the aspiration site (tip) at the ligament of Treitz. A marker perfusion site was located 20 cm proximal to the tip, and the third canal of the tube ended in the antral portion of the stomach. A separate single-lumen nasogastric tube (3 mm OD) was placed in the most dependent part of the stomach. The final position of the tubes was verified by fluoroscopy. With the subjects comfortably seated, an infusion of polyethylene glycol (PEG) 4000 (1.5 g PEG/100 ml, 0.154 mol/l saline) was begun through the proximal port of the duodenal tube at a rate of 1.5 ml/min. Duodenal contents were aspirated continuously during the experiments by means of a suction pump, which provided intermittent negative pressure. After an equilibration period, two premeal samples were collected from the duodenal tube in 15-min periods. Before meal administration the stomach was completely emptied by manual aspiration with a 50-ml syringe through the nasogastric and the proximal channel of the

triple-lumen tube. A 500-ml mixed liquid test meal (Ensure, Abott, Chicago, IL; diluted with 100 ml of distilled water) containing 16.8 g of protein, 13.4 g of fat, 53.4 g of carbohydrate, and a total caloric value of 400 kcal (254 mosmol/l, pH 7.0) was labeled with ^{99m}Tc-diethylenetriaminepentaacetic acid and infused intragastrically through the nasogastric tube over a standardized 7-min period.

Sample collection. Gastric samples (10 ml) were collected at the end of each 15-min period via the nasogastric tube by means of a 20-ml syringe. Duodenal samples were continuously siphoned on ice during 15-min periods after meal administration. Separate aliquots of gastric and duodenal juices were treated as follows. Native gastric and duodenal samples were used for analysis of pH, acid content, PEG, and ^{99m}Tc. For optimal storage conditions the pH of gastric and duodenal samples was adjusted to 4–5 for analysis of HGL. To prevent proteolytic inactivation, turkey egg white inhibitor (10 mg/ml) was added to gastric and duodenal samples (23) immediately after collection. Glycerol (50% vol/vol) was added to gastric samples for later pepsin measurements and to duodenal samples for HPL analysis. All duodenal samples were assayed within 1 mo for HGL and HPL activity. HGL showed no loss in activity after 1 and 2 mo when stored under the above conditions. Blood was sampled through a separate indwelling venous catheter at −30, −20, and −10 min and at 10, 20, 30, 45, 60, 90, 120, 150, and 180 min after meal administration. Blood samples were collected in 10-ml tubes containing 16 mg of EDTA and 8,000 U of aprotinin and were immediately centrifuged (4°C, 3,000 rpm, 15 min). Immediately after these different procedures, all samples were frozen on dry ice and stored at −20°C until they were assayed.

Experimental Protocol

Three experiments were performed in a randomized single-blind order in the six subjects. Each experiment consisted of a 60-min premeal period and a 180-min postmeal period. Placebo (500 ml of 0.9% saline), atropine (5 $\mu g \cdot kg^{-1} \cdot h^{-1}$), or loxiglumide (Rotta Research Laboratorium, Monza, Italy; 66 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ for 10 min, then 22 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ during the following 230 min) was administered by infusion pump through a separate indwelling intravenous catheter. The infusion protocol of loxiglumide has been shown to result in steady-state plasma concentrations of loxiglumide in the range of 150–250 μg/ml (34) while completely inhibiting pancreatic enzyme secretion and gallbladder emptying (14).

Determinations

Gastric and duodenal samples were analyzed for PEG concentrations (26) and ^{99m}Tc activities. Duodenogastric PEG reflux was absent or negligible. Gastric acid concentrations were measured by titration of 1-ml samples to pH 7.0 with 0.01 N NaOH using a pH-stat titration system (Metrohm, Herisau, Switzerland). Pepsin was determined in gastric juice by the method of Berstad (5) using human hemoglobin as substrate.

Lipase activity measurements. The activity of HGL was measured by the method of Borgström (7) using an automatic pH-stat titration system (Metrohm). The assay was performed in a reaction vessel containing 1.5 ml of a mechanically stirred emulsion prepared with 400 μl of tributyrin (T 8626, Sigma Chemical, St. Louis, MO) in 12 ml of buffer. The buffer contained 4 mM taurodeoxycholate, 2 mM tris(hydroxymethyl)aminomethane, 150 mM NaCl, and 0.2 mM $CaCl_2$ at pH 7. Titration of HGL activity was performed at pH 5.6 (specific activity = 318 U/mg purified HGL). The interassay variation (n = 36) for HGL measurements was 6.6%, and the intra-assay variation (n = 5) was 2.3%.

The specific activities of HGL and HPL measured at different pH values are shown in Fig. 1, demonstrating an assay optimum for HPL at pH 8.0 and for HGL at pH 5.6. The activity of HPL was measured at pH 8.0 (specific activity = 1,785 U/mg purified HPL) using 1.5 ml of an emulsion containing 1.5 ml of triolein (Fluka, Buchs, Switzerland) in 35 ml of buffer with 1 mM taurochenodeoxycholate, 9 mM taurocholate, 0.1 mM cholesterol, 1 mM phosphatidylcholine, 15 mg of bovine serum albumin per milliliter (free fatty acid free), 2 mM tris(hydroxymethyl)aminomethane, 100 mM NaCl, and 0.2 mM $CaCl_2$ (18). The interassay variation (n = 18) for HPL measurements was 6.4%, and the intra-assay variation (n = 5) was 1.1%.

In duodenal samples, HGL and HPL activity could be accurately discriminated. By lowering the pH of duodenal samples to 2 at 1 h before the HGL assay, HGL activity was slightly increased [104 ± 1% (SE), n = 8], whereas HPL was completely inactivated and not measurable under standard assay conditions (Fig. 2). Furthermore, HGL activity was shown not to be influenced by the proteolytic action of pepsin when pure pepsin was added to gastric juice during a 1-h incubation period at pH 2, confirming previous observations (29). The HPL determination was highly specific, inasmuch as HGL and carboxyl ester lipase are not active under HPL standard assay conditions using the long-chain triacylglycerol triolein as substrate (17). Furthermore, HPL was measured at its pH optimum of 8.0, whereas HGL was determined at its optimal pH of 5.6.

HGL and HPL activities are expressed in international units per milliliter (1 U = 1 μmol of fatty acids released per minute). Furthermore, concentrations of the two lipases are also expressed in milligrams of protein of the respective lipase.

Enzyme-linked immunosorbent assay of HGL. In addition to the measurement of enzyme activities, HGL was determined by a highly specific sandwich enzyme-linked immunosorbent assay (ELISA) in duodenal juice, as previously described in detail (4). Briefly, the ELISA was performed using an anti-HGL polyclonal antibody as captor antibody and a biotinylated monoclonal antibody (MAb 35) as the detector antibody. Antibody binding was read by optical density at 490 nm using a Dynatech MR 5000 spectrophotometer. By use of this sandwich ELISA procedure, the recovery of HGL added to duodenal samples was 94 ± 4%.

Radioimmunoassays of CCK and gastrin. Plasma CCK concentrations were measured by a sensitive and specific radioimmunoassay (21). Antibody T204 binds to all CCK peptides containing the sulfated tyrosyl region of CCK. The antibody shows <2% cross-reactivity to sulfated forms of

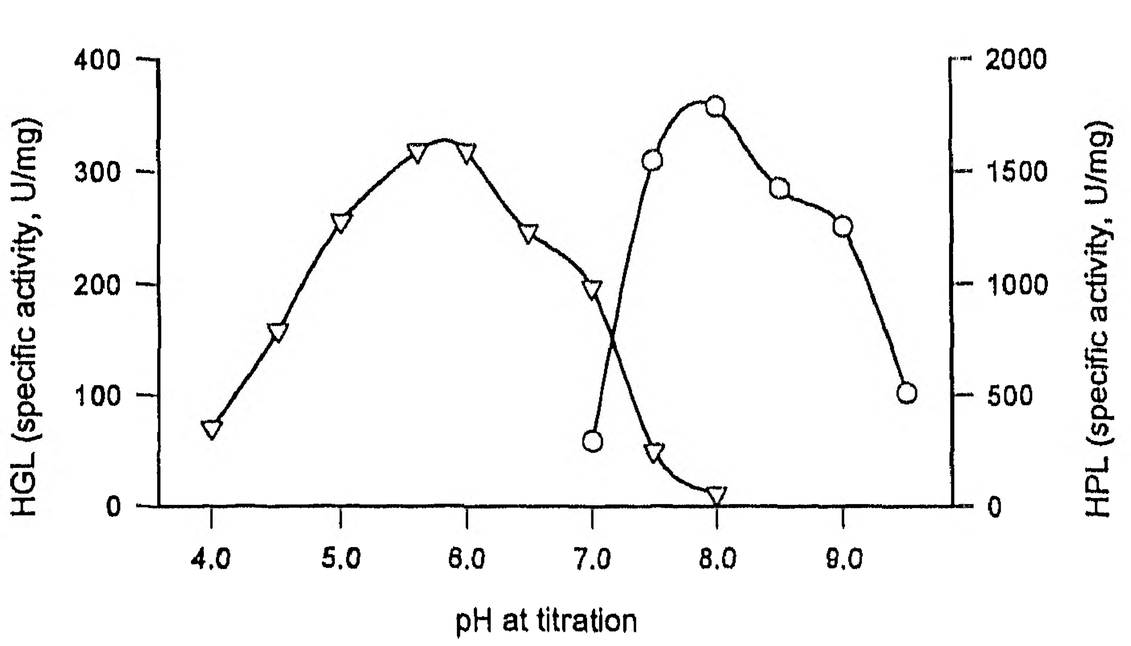

Fig. 1. Specific activity of purified human gastric lipase (HGL, ▽) and human pancreatic lipase (HPL, ○) in relation to pH used at titration. Points are means of ≥2 measured values.

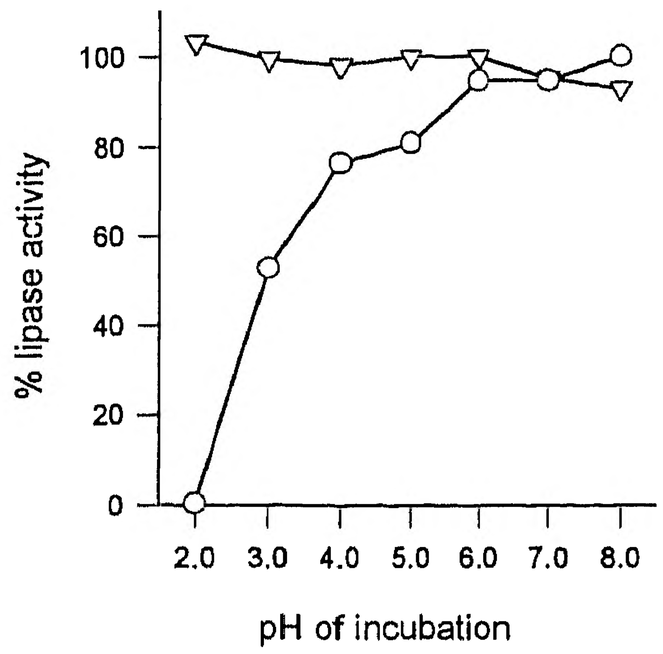

Fig. 2. Percentage of remaining activity of HGL (▽) and HPL (○) measured titrimetrically (at pH 5.6 for HGL and at pH 8.0 for HPL) 1 h after incubation at different pH values. Points are means of ≥2 measured values.

gastrin and no binding to nonsulfated gastrins. It does not cross-react with structurally unrelated gastrointestinal peptides. Synthetic CCK octapeptide (CCK-8) coupled to ^{125}I-hydroxyphenylpropionic acid succinimide ester (Bolton-Hunter reagent) was used as a standard. The detection limit of the assay was 0.5 pmol/l plasma, and the extraction recovery was 90 ± 2% when sulfated CCK-8 was added to hormone-free plasma. The intra- and interassay variations were 8% and 11%, respectively, at 2.6 pmol/l.

Plasma gastrin levels were measured by a sensitive and specific radioimmunoassay, as previously described in detail (6). The intra-assay variation was 7 ± 3% (mean ± SD, n = 20). All samples were analyzed in the same run.

Calculations and Statistical Analysis

Gastric meal emptying and gastric acid, HGL, and pepsin outputs were calculated according to previously published methods (24). Cumulated gastric acid, HGL, and pepsin outputs were calculated at the time when >90% of gastric contents had left the stomach. Secretory outputs of HGL and HPL passing the duodenal sampling site were calculated from the product of enzyme concentrations and flow rates. ^{99m}Tc recovery (marker given with the test meals corrected for the amount of ^{99m}Tc aspirated from the stomach) was 83 ± 5% (SE). Flow rates passing the duodenal sampling site were calculated on the basis of known infusion rates and PEG 4000 concentrations at the infusion and sampling ports. The emptying curves were fitted to a power exponential model, as described previously (11). The area under the curve (AUC) and half emptying time were calculated for each individual curve.

Values are means ± SE. Cumulated gastric acid, HGL, pepsin, and HPL outputs were calculated. Increments of CCK and gastrin concentrations were computed by subtracting the basal from the postprandial values, and integrated increments (AUC) were then calculated. Responses were compared by the Wilcoxon signed rank test. Differences were considered significant at $P < 0.05$.

RESULTS

Gastric Enzyme and Acid Secretion

There was no significant difference among basal duodenal outputs of HGL during placebo (0.9 ± 0.2 mg/15 min), loxiglumide (1.7 ± 0.5 mg/15 min), and atropine (0.5 ± 0.2 mg/15 min) infusion (Fig. 3). Postprandial gastric secretions of the two enzymes, HGL and pepsin, and of gastric acid reached a peak at 75–90 min after meal administration (Fig. 4). Loxiglumide markedly ($P < 0.03$) increased the secretion of HGL, pepsin, and gastric acid (Fig. 4, Table 1). Thus the infusion of loxiglumide caused a more than twofold increase in the cumulated postprandial HGL, pepsin, and acid secretion compared with placebo (Table 1).

Atropine almost completely inhibited gastric secretion of HGL, pepsin, and acid ($P < 0.03$; Fig. 4). The cumulated outputs of postprandial HGL secretion were markedly lower during atropine than during placebo infusion (18.4 ± 3.4 vs. 65.2 ± 6.2 mg for 120 min, P = 0.028). In parallel, atropine distinctly decreased gastric outputs of pepsin (128.4 ± 28.3 vs. 397.6 ± 53.2 mg for 120 min, P = 0.028) and acid (20.8 ± 3.5 vs. 49.0 ± 7.5 mmol for 120 min, P = 0.028).

HGL secretion as determined in duodenal aspirates showed a late maximal rise after 90 and 120 min and was markedly augmented by loxiglumide compared with placebo (Fig. 3). Atropine almost completely inhibited HGL secretion. Cumulated HGL outputs as measured by lipase activity assay and ELISA were markedly increased by loxiglumide and decreased by atropine (Table 2). Thus there was an excellent correlation of HGL outputs measured by the two different methods (r = 0.7453, $P < 0.001$).

Duodenal HPL Outputs

Loxiglumide markedly suppressed basal HPL outputs (1.7 ± 0.3 mg/15 min, $P < 0.05$). Atropine also

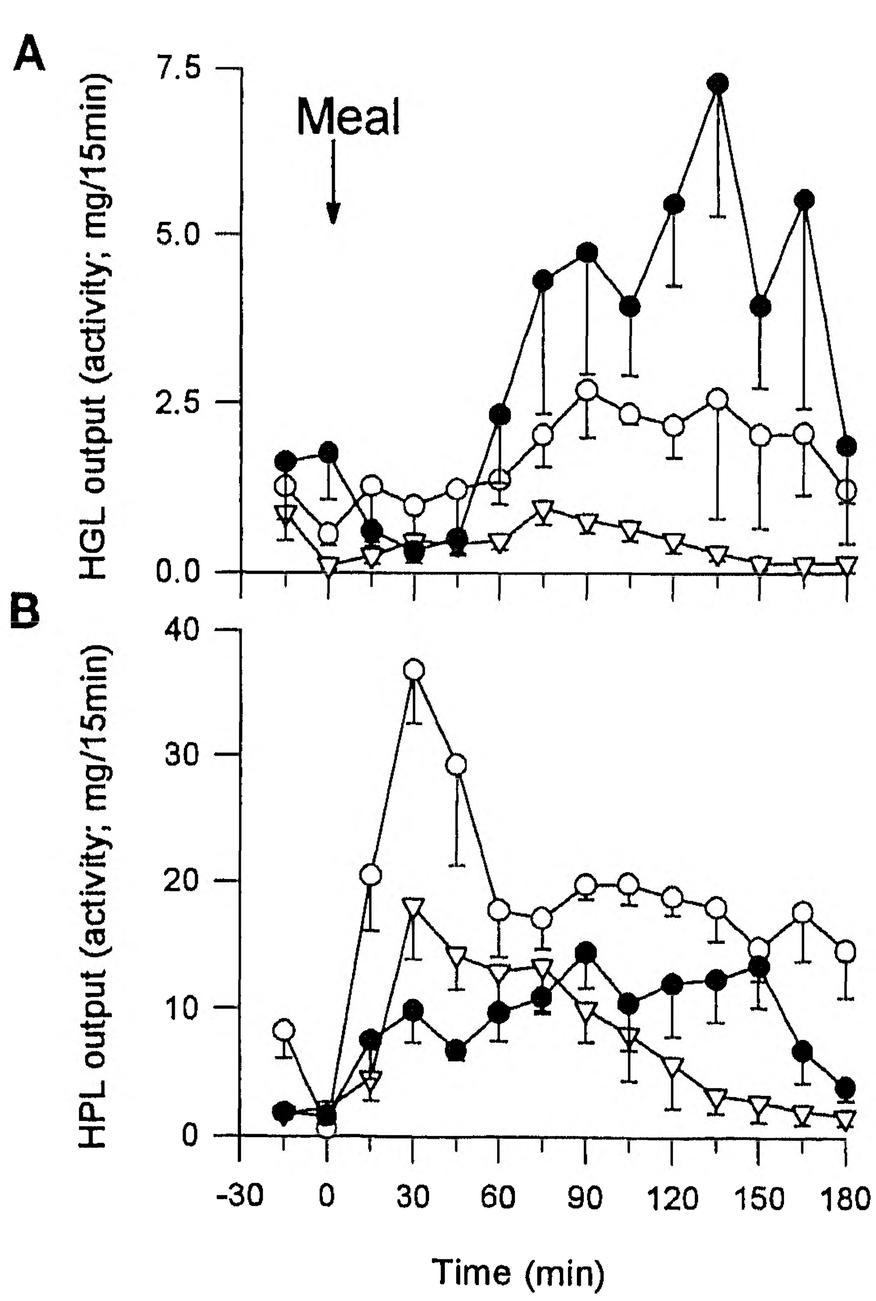

Fig. 3. Duodenal outputs of HGL and HPL after an intragastric mixed meal during infusion of placebo (○), atropine (▽, 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$), and loxiglumide (●, 22 $\mu mol \cdot kg^{-1} \cdot h^{-1}$). Values are means ± SE of 6 subjects.

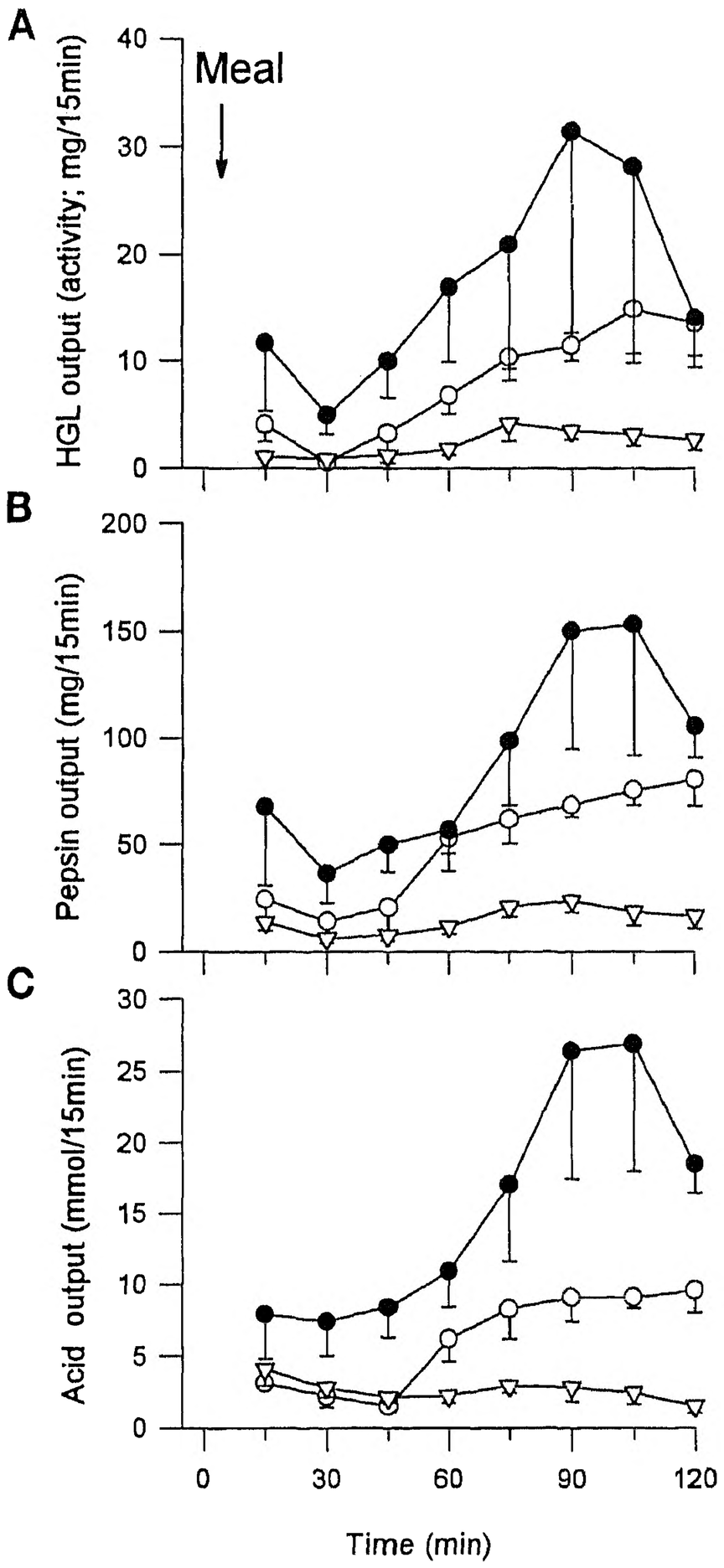

Fig. 4. Gastric outputs of HGL, pepsin, and acid after an intragastric mixed meal during infusion of placebo (○) atropine (▽, 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$), and loxiglumide (●, 22 $\mu mol \cdot kg^{-1} \cdot h^{-1}$). Values are means ± SE of 6 subjects.

reduced basal HPL outputs (2.0 ± 0.9 mg/15 min, $P < 0.03$) compared with placebo (8.5 ± 2.5 mg/15 min; Fig. 3*B*). The postprandial HPL secretion is shown in Fig. 3. HPL secretion reached a maximum 30 min after meal administration during infusion of placebo; loxiglumide and atropine suppressed this early postprandial response to a similar degree by >50%. Accordingly, the

Table 1. *Effects of loxiglumide on cumulative postprandial gastric outputs (75 min) of HGL, pepsin, and HCl secretion*

Experiment	HGL, mg	Pepsin, mg	HCl, mmol
Placebo	31.5 ± 8.7	174 ± 43	21.3 ± 4.2
Loxiglumide	64.7 ± 22.4*	309 ± 73*	51.6 ± 9.6*

Values are means ± SE. HGL, human gastric lipase. *$P < 0.03$ vs. placebo.

Table 2. *Effects of loxiglumide and atropine on cumulated postprandial duodenal outputs (180 min) of HGL and HPL*

Experiment	HGL Activity, mg	HGL ELISA, mg	HPL Activity, mg
Placebo	22.0 ± 5.8	24.7 ± 9.1	245 ± 22
Loxiglumide	40.9 ± 7.1*	83.0 ± 31.4*	119 ± 17*
Atropine	5.3 ± 1.1*	5.4 ± 1.4*	97.5 ± 26.2*

Values are means ± SE. HPL, human pancreatic lipase; ELISA, enzyme-linked immunosorbent assay. *$P < 0.03$ vs. placebo.

cumulated outputs of HPL were decreased distinctly by atropine and loxiglumide (Table 2).

Gastric Emptying

Loxiglumide distinctly accelerated gastric emptying of the mixed liquid meal ($P < 0.03$; Fig. 5). AUC (3,703 ± 184 vs. 5,720 ± 457 pM·min, $P < 0.03$) and half emptying time (29.7 ± 1.5 vs. 51.1 ± 4.7 min, $P < 0.03$) were considerably decreased by loxiglumide compared with placebo. Atropine slowed gastric emptying slightly (AUC = 6,911.8 ± 624.2 vs. 5,720.3 ± 456.5 pM·min, $P < 0.05$) without a significant delay of the half emptying time (63.4 ± 5.7 vs. 51.1 ± 4.7 min, $P = 0.075$).

Plasma Gastrin and CCK

Basal plasma gastrin concentrations remained unchanged during the infusion of loxiglumide (7.3 ± 1.8 and 8.2 ± 2.1 pM without and with loxiglumide, respectively) and atropine (13.8 ± 4.9 pM, P = NS). Maximal plasma levels were measured 10–20 min after meal administration during placebo (26.8 ± 6.4 pM), loxiglumide (46.2 ± 10.3 pM), and atropine (37.7 ± 11.6 pM) infusions (Fig. 6). Postprandially, integrated gastrin values (AUC) were markedly increased by loxiglumide compared with placebo (3,234 ± 570 vs. 1,982 ± 419 pM·min, $P < 0.03$) and were significantly augmented by atropine (2,743 ± 573 pM·min, $P < 0.05$).

Basal plasma CCK concentrations were not altered by the infusion of loxiglumide (2.3 ± 0.4 pM) and atropine (2.2 ± 0.3 pM) compared with placebo (2.7 ±

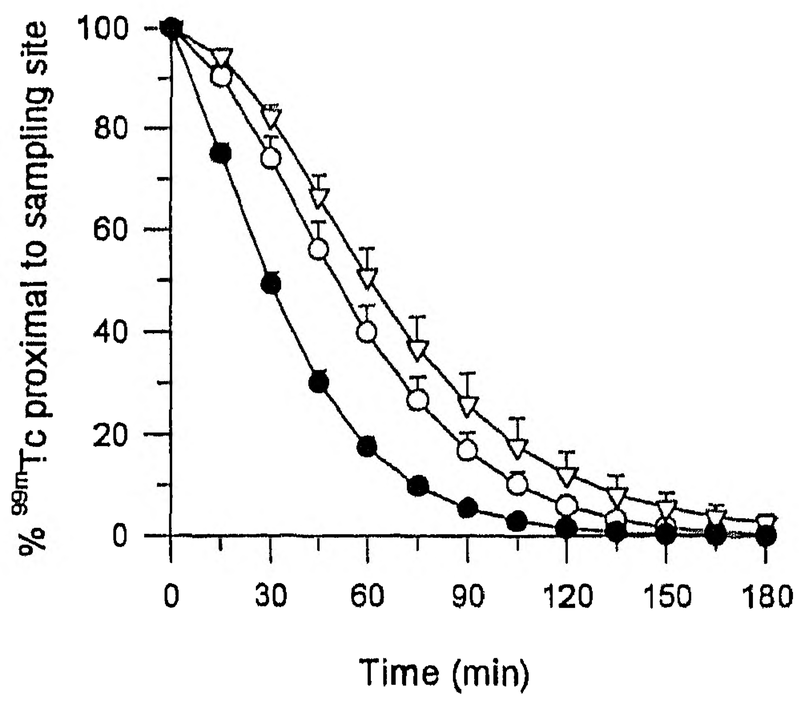

Fig. 5. Gastric emptying of a ^{99m}Tc-labeled liquid mixed meal during infusion of placebo (○) atropine (▽, 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$), and loxiglumide (●, 22 $\mu mol \cdot kg^{-1} \cdot h^{-1}$). Values are means ± SE of 6 subjects.

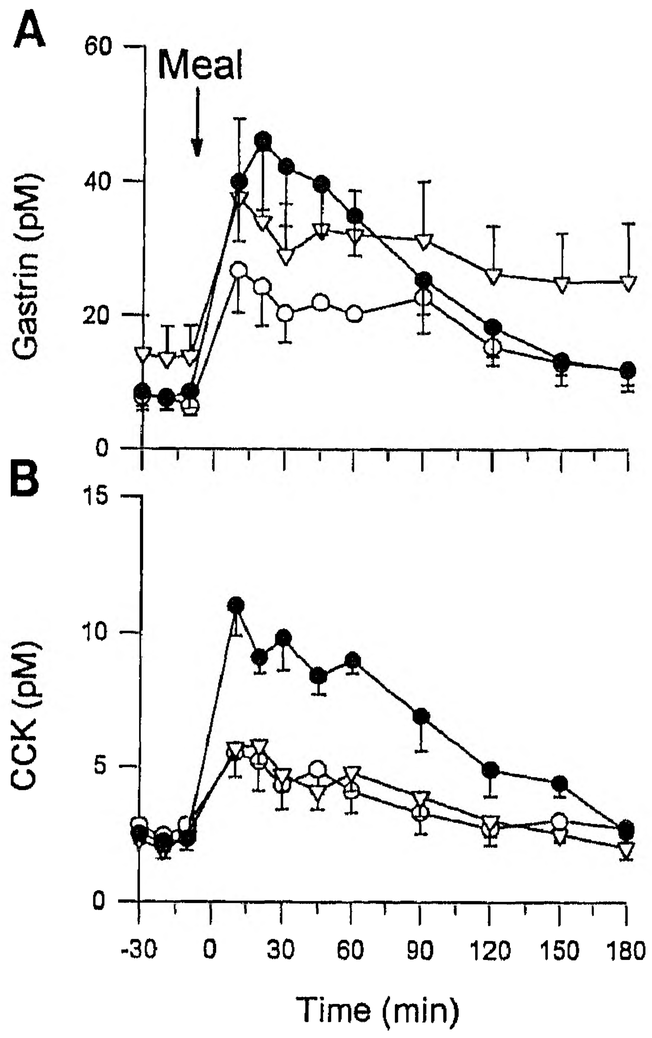

Fig. 6. Plasma cholecystokinin (CCK) and gastrin before and after an intragastric mixed meal during infusion of placebo (○) atropine (▽, 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$), and loxiglumide (●, 22 $\mu mol \cdot kg^{-1} \cdot h^{-1}$). Values are means ± SE of 6 subjects.

0.4 pM). Maximal increments were reached 10 min after meal instillation during placebo (5.5 ± 0.9 pM), loxiglumide (11.0 ± 1.1 pM), and atropine (5.7 ± 0.7 pM) infusion followed by a gradual decline (Fig. 6). Postprandially, loxiglumide markedly increased integrated CCK values (AUC) compared with placebo (765 ± 98 vs. 173 ± 117 pM·min, $P < 0.03$), whereas atropine had no effect on CCK release (270 ± 55 pM·min).

DISCUSSION

HGL has recently been identified to be one of the key enzymes of fat digestion in humans (1, 8). However, the regulation of its secretion has remained poorly understood. We have demonstrated that gastric and pancreatic lipolysis are regulated by CCK and cholinergic mechanisms in a different and specific way. Thus the infusion of the specific CCK-A receptor antagonist loxiglumide stimulated postprandial secretion of HGL, pepsin, and acid while inhibiting pancreatic secretion of HPL. Low-dose atropine inhibited gastric and pancreatic enzyme secretions. Furthermore, we found a parallel secretion of the two gastric enzymes, HGL and pepsin, both being inhibited by CCK and stimulated by the cholinergic system. In addition, we confirmed in this study the inhibitory effect of postprandially released CCK and atropine on gastric meal emptying.

Loxiglumide markedly inhibited postprandial HPL secretion while distinctly increasing HGL and pepsin secretion, indicating complementarity in the regulation of the two main lipolytic enzymes, HGL and HPL. In contrast, in a previous study with exogenously administered intravenous CCK, no regulatory effect of CCK on HGL secretion was found (30). Thus, by applying the specific CCK-A antagonist loxiglumide, we could demonstrate, to our knowledge for the first time, a marked stimulation of postprandial gastric secretion of the two gastric enzymes, HGL and pepsin, consistent with a negative-feedback regulation by CCK. Furthermore, our experiments outline the presence of a parallel postprandial secretion of HGL, pepsin, and gastric acid commonly regulated by CCK and cholinergic mechanisms. These findings are in line with previous studies showing a parallel secretion of HGL and pepsin after exogenous injection of pentagastrin and a common regulation of pepsin and gastric acid in humans (20, 39). In analogy to the parallel secretion of digestive enzymes by the pancreas, the postprandial gastric secretion of HGL and pepsin may be explained by their colocation in human gastric chief cells, as recently shown by immunocytochemical studies (28). The inhibition of postprandial gastric acid outputs by CCK as shown in our study confirms previous results obtained in humans by gastric pH-metry and by measurement of stimulated acid output (22, 35).

The mechanisms by which CCK exerts its regulatory action on postprandial gastric enzyme and acid secretion are poorly understood. In our study, parietal and chief cell secretion were stimulated by loxiglumide, although these two gastric mucosal cells differ considerably in their cell receptors for CCK and gastrin. CCK-A receptors of the pancreatic type, absent in parietal cells, were identified in the rabbit chief cell by cDNA cloning (19). Whereas the presence of CCK-A receptors has been demonstrated in a model of gastric chief cells, their functional relevance regarding HGL and pepsin secretion remains unclear. CCK is a full gastrin agonist that acts through a CCK-B-type gastrin receptor on parietal cells, exerting only a weak agonist activity in vivo and potently inhibiting pentagastrin-stimulated gastric acid secretion (37). In contrast to the inhibitory action on gastric enzyme and acid secretion in vivo, CCK-8 potently stimulates HGL secretion in rabbit and human gastric glands (10, 13). Thus the different CCK-type receptors on gastric chief (CCK-A) and parietal cells (CCK-B) do not indicate a common regulatory mechanism through CCK-A receptors located in the gastric gland. However, potentially more sensitive intermediary neuroceptors for CCK, located in capsaicin-sensitive vagal afferent fibers in the gastric wall, may mediate the inhibitory effect of CCK on gastric secretion and emptying and may explain the discrepancy between in vitro stimulation and in vivo inhibition of gastric secretion (32). Other mechanisms, such as the paracrine secretion of somatostatin, might be candidates for this regulation, inasmuch as somatostatin inhibits secretion of parietal and gastric chief cells (25). In vitro models show that somatostatin interacts with a specific somatostatin subtype receptor (SSTR-2) on gastrin and histamine cells in humans (42). In vivo experiments in dogs confirm an inhibitory effect of somatostatin analogs on postprandial gastric acid secretion through the same receptor subtypes (15).

Our study design enabled us to measure postprandial HGL secretion and to investigate simultaneously the duodenal lipolytic activity of HGL and HPL. Moreover, HGL outputs were measured by a titrimetrical

and an enzyme-linked assay to quantify the activity and the concentration of HGL. The two assay methods showed an excellent correlation, with differences only at high secretory rates of HGL. This is probably due to a partial inactivation of HGL by proteases, resulting in lower HGL activities than quantified by ELISA. Correspondingly, higher amounts of HGL were measured titrimetrically in gastric contents than in the duodenum with its high concentrations of proteases. Confirming previous observations (8), we found that the maximal HGL output was different 45 min after meal ingestion at the gastric and the duodenal site, which can be explained by a late and incomplete emptying of the enzyme-rich gastric mucus.

The administration of low-dose atropine resulted in decreased outputs of HPL in the duodenum and of HGL at the duodenal and gastric sampling sites. Atropine reduced HPL activity by ~50%, similar to previously reported experiments with Lundh meals (16). In another study an almost complete inhibition of HPL was obtained with the same dose of atropine (5 $\mu g \cdot kg^{-1} \cdot h^{-1}$) after intraduodenal perfusion of the test meal (3). The cholinergic regulation of gastric secretion of acid and pepsins was previously studied in humans and dogs (12). This study demonstrates that HPL and HGL are regulated by muscarinic pathways, the latter in parallel to gastric pepsin and acid secretion, confirming previous observations in vagotomized patients (39) and studies on gastric acid and pepsin secretion (12, 38). Thus the present study confirms and extends these previous results demonstrating cholinergic inhibition of gastric acid, pepsin, HGL, and HPL secretion (39).

We and other groups have shown previously that CCK potently inhibits gastric emptying in animals and humans (9, 14). In the present study, CCK-A receptor blockade with loxiglumide resulted in a marked acceleration of gastric emptying of a mixed liquid meal, confirming the important role of CCK in gastric emptying. Furthermore, our study showed that atropine at a low dose of 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ did not influence heart rate but significantly delayed gastric emptying. From previous studies in humans, atropine was known to inhibit gastric emptying in a dose-related fashion at >3 $\mu g \cdot kg^{-1} \cdot h^{-1}$ and was shown to act in the animal model by abolishing the activation of the antropyloroduodenal region and the decrease in fundic tone relaxation (27, 33). However, besides cholinergic neurons, vagal stimulation of gastric emptying implies adrenergic ganglia and noncholinergic nonadrenergic enteric nerves with release of nitric oxide and vasoactive intestinal peptide as primary mediators that may partially substitute for a cholinergic blockade by atropine.

Measurements of gastrin release showed a marked increase during CCK-A receptor blockade with loxiglumide. These results are in line with previous studies showing augmented gastrin release by loxiglumide after oral meal ingestion (35) and confirm an important role of CCK in the regulation of postprandial gastrin secretion. The administration of low-dose atropine induced an increase in gastrin secretion that was in contrast to the marked inhibition of gastric acid secretion. The inverse relationship of acid inhibition and gastrin stimulation during atropine infusion was previously reported in humans and may be explained by a suppressed somatostatin activity, resulting in a secondary increase of gastrin release (12, 33). Studies with the selective gastrin receptor antagonist YM-022 in rats showing no inhibition of bethanechol- and histamine-stimulated acid secretion question the importance of gastrin in the regulation of gastric acid secretion (31). Further studies with CCK-B/gastrin receptor antagonists in humans are needed to clarify the role of endogenous gastrin in gastric acid secretion and gastric fat digestion.

CCK release was unaffected by atropine and considerably increased during loxiglumide infusion. The phenomenon of enhanced CCK release during CCK-A receptor blockade has been previously reported (14) and is explained by the inhibition of gallbladder emptying and, in consequence, the interruption of a bile acid-mediated feedback mechanism on CCK release. This hypothesis was recently confirmed in a study with different bile salt binders in humans, demonstrating that bile salt sequestration, but not protease inactivation, enhances plasma CCK levels stimulated by bombesin in humans (40).

In conclusion, the postprandial gastric secretion of lipase, pepsin, and acid occurs in parallel and is stimulated by cholinergic mechanisms while being inhibited by CCK. HPL secretion is stimulated by cholinergic mechanisms and CCK. Thus the regulation of postprandial fat digestion by CCK consists of a positive pancreatic stimulation and a negative-feedback mechanism on HGL secretion. The main mechanisms postulated by which CCK coordinates gastric and pancreatic fat digestion include vagal pathways and the stimulation of paracrine somatostatin secretion.

We thank Dr. Massimo D'Amato (Rotta Research Laboratories, Monza, Italy) for providing the loxiglumide.

This work was supported by Swiss National Science Foundation Grant 32–33723.92.

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Received 4 November 1996; accepted in final form 11 April 1997.

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